

Serum Vitamin E and Malondialdehyde (MDA) Levels in Cases With Head and Neck Cancer

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Summary : Lipid peroxidation is a complex chain reaction and a very important process in free radical pathology. Malondialdehyde (MDA) is one of the aldehydic end products of this process. These end products can cause DNA damage, and lipid peroxidation-induced damage to DNA may be involved in carcinogenesis. Vitamin E is the most effective lipid soluble antioxidant, and vitamin E protect critical cellular structures against damage from oxygen free radicals and reactive products of cancer initiation and promotion. In this study, malondialdehyde levels as an index of lipid peroxidation, antioxidant vitamin E, cholesterol and triglyceride levels were determined in sera obtained from 54 patients newly diagnosed with head and neck cancer (Larynx carcinoma, n=35; Others, n= 19) and healthy subjects (n=54). The effects of age, sex, quetelet index (kg/m²) and stages of cancer upon these parameters were evaluated. In addition, any possible correlation between MDA and vitamin E levels was investigated. MDA levels were measured by the modified Yoshioka's TBA method and vitamin E levels were measured by Martinek's method. MDA levels were significantly higher in the patient group (9.09±6.03 µmol/ L) when compared with control group (3.87±0.72 µmol/ L) (p<0.001). Vitamin E levels of cases (0.36±0.15 mg/dL) were lower than those of controls (0.81±0.07mg/dL), (p<0.001). In our study cholesterol and triglyceride levels of cases were similar to controls. There were no statistically significant differences between cases and controls in terms of these parameters. Except for age and quetelet index, no statistically significant differences were found among stages and the subgroups of sex, in terms of MDA and Vit.E levels. In this study, according to alcohol intake and cigarette smoking, MDA and vitamin E levels could not be evaluated because of the fact that all the cases were ex-smokers and also were not drinking alcohol. There was no correlation between MDA levels and vitamin E levels in both cases and controls. The results of this study indicate that there is an increase in lipid peroxidation and a decrease in vitamin E levels in carcinogenesis.

Key words: Head and neck cancer, lipid peroxidation, malondialdehyde(MDA), antioxidant, vitamin E

Received : 29.4.1998

Revised : 2.7.1998

Accepted : 2.7.1998

Baş ve Boyun Bölgesi Kanserli Hastalarda Serum Vitamin E ve Malondialdehit (MDA) Düzeyleri

Özet : Lipid peroksidasyonu kompleks bir zincir reaksiyonudur ve serbest radikal patolojisinde oldukça önemli bir prosestir. Malondialdehit (MDA) bu prosesin aldehit yapıdaki son ürünlerinden biridir. Bu son ürünler DNA hasarı oluşturabilir ve lipid peroksidasyonu ile indüklenen DNA hasarı karsinojeneze neden olabilir. Vitamin E lipitte çözünen en etkili antioksidan olup kritik hücreyel yapıları oksijen serbest radikallerinin ve kanserin başlaması ve ilerlemesinde oluşan reaktif ürünlerin hasarına karşı korumaktadır. Bu çalışmada, yeni teşhis konmuş baş ve boyun bölgesi kanserli (larinks karsinom, n=35; diğerleri, n=19) 54 hasta ve sağlıklı kişilerden (n=54) elde edilen serumlarda; lipid peroksidasyonunun indeksi olarak malondialdehit, antioksidan E vitamini, kolesterol ve trigliserit düzeyleri saptanmıştır. Bu parametreler üzerine yaş, seks, quetelet indeksi (kg/m²) ve kanser evresinin etkileri incelenmiştir. Ayrıca MDA ile E vitamini düzeyleri arasında bir korelasyon olup olmadığı araştırılmıştır. MDA düzeyleri modifiye Yoshioka TBA metodu ve E vitamini düzeyleri Martinek metodu ile ölçüldü. Kontrol grubu ile (3.87±0.72 µmol/L) karşılaştırıldığında MDA düzeyleri hasta grubunda (9.09±6.03 µmol/L) önemli derecede daha yüksekti (p<0.001). Hastaların (0.81±0.07 mg/dL) daha düşüktü (p<0.001). Çalışmamızda hastaların kolesterol ve trigliserit düzeyleri kontrollerle benzerlik göstermekteydi. Hasta ve kontrol arasında her iki parametre yönünden istatistiksel olarak anlamlı bir farklılık bulunamadı. Yaş ve quetelet indeksi hariç, seks ve kanser evreleri alt grupları arasında MDA ve E vitamini düzeylerine göre istatistiksel olarak önemli farklılık bulunamadı. Bu çalışmada alkol tüketimi ve sigara kullanımına göre MDA ve E vitamini düzeyleri hastaların sigarayı bırakmış olmaları ve alkol kullanmamaları nedeniyle değerlendirilememiştir. Hem hastaların, hemde kontrollerin MDA düzeyleri ve E vitamini düzeyleri arasında korelasyon bulunamadı. Bu çalışmanın sonuçları kanserde lipid peroksidasyonunda artış ve vitamin E düzeylerinde azalma olduğunu göstermektedir.

Anahtar kelimeler: Baş ve boyun bölgesi kanseri, lipid peroksidasyonu, malondialdehit (MDA), antioksidan, vitamin E.

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INTRODUCTION

Free radical-induced damage is etiologically implicated in many diseases including cancer, aging and many degenerative diseases¹. There is increasing evidence to support the involvement of free radical reactions in cancer^{2,3}. Lipid peroxidation is a complex chain reaction and a very important process in free radical pathology. In this process, polyunsaturated fatty acids (PUFAs) in the phospholipids of cellular membranes undergo reaction with reactive free radicals to yield various reactive species such as lipid peroxy radicals, alkoxy radicals and cytotoxic aldehydes. Malondialdehyde is also one of the aldehydic end products of this process. The major source of malondialdehyde is polyunsaturated fatty acids and it is formed during the last stages of the breakdown of endoperoxides⁴. These products can produce DNA damage, and lipid peroxidation-induced damage to DNA may be involved in carcinogenesis^{5,6}.

The biological effects of free radicals are controlled in vivo by a wide range of antioxidants such as vitamins E and C, carotenoids, and antioxidant enzymes. Vitamin E, the most effective chain-breaking lipid soluble antioxidant, is thought to be an important structural component of biological membranes and is believed to act as a free radical scavenger in lipid peroxidation. Vitamin E protects critical cellular structures against damage from oxygen free radicals and reactive products of cancer initiation and promotion. Vitamin E inhibits this damage mainly through its antioxidant function by eliminating oxygen free radicals and thus decreasing DNA damage^{7,8}. Recent studies indicate that vitamin E exerts significant anticarcinogenic activity and plays an important role in cancer prevention and treatment. These studies also suggest that vitamin E may prevent or reduce oral and esophageal carcinogenesis and that a significant lowering of oral cavity cancer incidence by as much as 50% is achieved with the use of supplemental vitamin E⁹⁻¹³.

A number of epidemiologic studies have examined cancer in relation to serum lipids¹⁴⁻¹⁶. Although several authors propose that hypocholesterolemia is a predisposing factor for cancer development, no causative relation has been established^{15,17}. Some authors believe that hypocholesterolemia is, in fact,

the result rather than the cause of cancer, and pre-clinical cancer in some way lowers serum cholesterol^{18,19}.

In this study, we aimed to determine vitamin E, malondialdehyde (MDA), cholesterol and triglyceride levels in patients with head and neck cancer to confirm the consistency of previous evidence on the relationship between serum antioxidant, and malondialdehyde levels in cancer and to evaluate cholesterol and triglyceride levels of cases and controls.

MATERIALS AND METHODS

In this study, malondialdehyde levels as an index of lipid peroxidation and vitamin E levels were determined in sera obtained from healthy subjects (n=54) and patients with clinically and histopathologically confirmed diagnosis of larynx carcinoma (n=35) and other types of head and neck cancer (total n=19; nasopharynx n=3, hypopharynx n=3, lowerlip n=3, oral cavity n=4, maxilla n=2, antrum n=2, tongue n=2). All blood samples were collected in the fasting state and blood samples of the cases were taken before the treatment. Sera were separated and stored at -20°C until analysis. Clinical staging of patients was done in accordance with the TNM classification for head and neck cancer (defined by American Joint Committee, 1988). The effects of age, sex, quetelet index [weight (kg) / height (m²)] and stages of cancer on these parameters were evaluated. Any correlation between MDA and vitamin E levels was investigated. Mean line characteristics of all subjects involved in this study were shown in Table 1. Serum MDA levels were measured spectrophotometrically at 532 nm by the Modified Yoshioka's TBA method²⁰. The coefficient of variation (CV) for MDA determinations was 6.33 % for intra-day precision and mean recovery was found to be 93.80 %. Serum Vitamin E levels were measured spectrophotometrically at 520 nm by Martinek's Method²¹. Intra-day precision of these method was 1.03-2.7 % (CV) and mean recovery was found to be 96±75%. Assay of serum cholesterol and triglyceride were performed enzymatically using Sclavo diagnostic kits (Sclavo Diagnostic Siena, Italy).

Statistical analysis was done using Student's t test,

Table 1 : Mean Line Characteristics of All Subjects

	LARYNX CANCER n=35 X±S.D	OTHER TYPES OF HEAD AND NECK CANCER n=19 X±S.D	TOTAL HEAD AND NECK CANCER n=54 X±S.D	CONTROL n=54 X±S.D	P
MDA µmol/L	10.25±6.94*	6.98±2.95*	9.09±6.03*	3.87±0.72	< 0.001
VITAMIN E mg/dL	0.36±0.02*	0.36±0.18*	0.36±0.15*	0.81±0.07	< 0.001
TRIGLYCERIDE mg/dL	134.28±61.94	128.11±43.88	133.96±55.86	138.24±66.96	> 0.05
CHOLESTEROL mg/dL	179.57±41.87	176.63±37.06	179.74±41.52	185.43±30.53	> 0.05

* p<0.001 for the difference between two means by student's t test compared to the control

Variance analysis and Regression analysis in the Instat statistic programs.

RESULTS AND DISCUSSION

The possible role of antioxidant vitamins in the prevention of cancer has been the focus of considerable research, and many reports on the relationship between micronutrients and cancer have been published. Previous hospital- and population-based case-control studies of oral and pharyngeal cancer showed that use of vitamin E appeared to exert a protective effect, and vitamin E supplement was also associated with a significantly reduced risk of

cancer^{10,13}. Results of another study in which phospholipids, cholesterol, malondialdehyde and antioxidant vitamin E levels were determined in buccal mucosa suggests that an increase in lipid peroxidation occurs with the tumour promotion process but this lipid peroxidation declines when the tumour appears to be preceded by an increase in cholesterol relative to phospholipids and an increase in vitamin E²².

The mean serum MDA, vitamin E, triglyceride and cholesterol levels of the patient groups with head and neck cancer and the healthy control group is shown in Table 2. Subjects with head and neck can-

Table 2 : Mean values of Serum MDA, Vitamin E, Cholesterol and Triglyceride in Cases and Controls

		CASES			CONTROLS		
		n	MDA µmol/L X±S.D	Vit.E mg/dL X±S.D	n	MDA µmol/L X±S.D	Vit.E mg/dL X±S.D
AGE	< 50	8	7.1±2.8	0.24±0.1**	25	3.7±0.79	0.81±0.08
	50-60	26	10.26±7.23	0.37±0.14	13	3.87±0.66	0.77±0.08
	> 60	20	8.93±4.91	0.39±0.18	16	3.97±0.71	0.83±0.06
SEX	Female	6	8.83±3.52	0.39±0.06	10	4.07±1.04	0.83±0.09
	Male	48	8.72±5.30	0.36±0.17	44	3.82±0.65	0.80±0.07
QUETELET INDEX	< 20	10	7.68±5.62	0.27±0.12	21	3.66±0.72	0.71±0.05*
	20-25	26	7.69±3.62	0.40±0.18	21	3.78±0.64	0.83±0.09
	> 25	18	10.70±6.31	0.33±0.12	12	4.02±0.81	0.89±0.07
SMOKING HABITS	Ex-smoker	54	9.09±6.03	0.36±0.15	54	3.87±0.72	0.81±0.07
ALCOHOL INTAKE	No	54	9.09±6.03	0.36±0.15	54	3.87±0.72	0.81±0.07
STAGE	Stage I	9	7.84±5.2	0.37±0.15	—	—	—
	Stage II	18	8.26±4.4	0.29±0.14	—	—	—
	Stage III	22	10.55±5.2	0.37±0.14	—	—	—
	Stage IV	5	6.94±6.2	0.43±0.14	—	—	—

** p<0.05 when compared with the group of age >60

* p<0.001 when compared with the groups of quetelet 20-25 and quetelet >25

cer had significantly higher MDA values (9.09 ± 6.03 $\mu\text{mol/L}$) than the control subjects (3.87 ± 0.72 $\mu\text{mol/L}$) ($p < 0.001$). When the patients were classified as larynx cancer group and other type of head and neck cancer, MDA values of patients with larynx cancer was higher (10.25 ± 6.94 $\mu\text{mol/L}$) than those of latter group (6.98 ± 2.95 $\mu\text{mol/L}$), and this difference was statistically significant ($p < 0.001$). Also vitamin E levels of cases (0.36 ± 0.15 mg/dL) were lower than those of the control group (0.81 ± 0.07 mg/dL) ($p < 0.001$), but levels were similar in both groups so that serum vitamin E values showed no difference between larynx cancer group and other types of head and neck cancer group. When we analysed the effects of age, sex, quetelet index (kg/m^2) and stages of cancer on MDA and vitamin E levels, no statistically significant differences were found among these groups, except for age and quetelet index.

According to age, there were statistically significant differences in terms of vitamin E levels between subgroups of age < 50 (0.24 ± 0.1 mg/dL) and subgroups of age > 60 (0.39 ± 0.18 mg/dL) in patients ($p < 0.05$). Vitamin E levels of controls with a quetelet index of < 25 were significantly lower than those of controls with a quetelet index of between 20-25 and those of controls with a quetelet index of > 25 . In addition, there was no correlation between MDA levels and vitamin E levels in both cases and controls ($r = 0.01$, $r = 0.06$, respectively).

Alcohol intake and cigarette smoking are established risk factors, for oral and pharyngeal cancer, and smoking was found to be associated with reduced serum levels of vitamin E²³. MDA and Vitamin E levels could not be evaluated according to alcohol intake and cigarette smoking due to the fact that all the persons taking part were ex-smokers and did not have any drinking habits.

Although a number of reports have been published indicating that there may be a relationship between low serum cholesterol levels in humans and increased cancer morbidity and mortality, there was no consistent or convincing evidence of an increased risk of cancer for persons with low cholesterol^{24,25}. Results obtained from these studies are inconsistent between sexes, among sites of cancer, and among investigators and/or study populations, and no mechanism is yet apparent. In our studies,

serum cholesterol (179.74 ± 41.52 mg/dL) and triglyceride (133.96 ± 55.86 mg/dL) levels were lower than those of controls (185.43 ± 30.53 ; 138.24 ± 66.96 mg/dL , respectively), but this difference was not statistically significant.

Although the antioxidant mechanism of vitamins and the role of lipid peroxidation in carcinogenesis has been investigated for years and several mechanisms have been proposed, there are still conflicting opinions. Free radicals are strongly reactive oxygen species in the tissues and they can be formed endogenously by normal metabolic processes or exogenously by factors external to the body. They have the ability to bound to most normal cellular components such as polyunsaturated fatty acids of cellular membranes. These chemical reactions lead directly to cell and tissue damage and are thus thought to initiate and/or promote cancer. The natural protectors against lipid peroxidation in serum are antioxidant vitamins such as vitamin E and C. Reduced antioxidant defence status of plasma might result in increased peroxidation of cell membrane lipids and hence increased concentrations of lipid peroxides. For this reason vitamin E is important in maintaining efficient inhibition of lipid peroxidation^{8,26}. This study indicates that there is an increase in lipid peroxidation and decrease in vitamin E levels in patients with head and neck cancer with regard to controls. The findings of this study together with our previous studies²⁷⁻²⁸ and many other previous studies provides further evidence that α -tocopherol may be a promising agent in the chemoprevention of cancer. We believed that our data will be useful for further epidemiological studies.

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